

FIG. I Sequence of CDRs of hTNF40

- H1 DYGMN (SEQ ID NO:1)
- H2 WINTYIGEPIYYDDFKG (SEQ ID NO:7)
H2' WINTYIGEPIYYADSVKG (SEQ ID NO:2)
- H3 GYRSYAMDY (SEQ ID NO:3)

- L1 KASQNVGTNVA (SEQ ID NO:4)
- L2 SASFLYS (SEQ ID NO:5)
- L3 QQYNIYPLT (SEQ ID NO:6)

FIG.2 Murine VI Sequence of hTNF40 (SEQ ID NO: 99)

GAC ATT GTG ATG ACC CAG TCT CAA AAA TTC ATG TCC ACA TCA GTA GGA GAC AGG	50
CTG TAA CAC TAC TGG GTC AGA GTT TTT AAG TAC AGG TGT AGT CAT CCT CTG TCC	
D I V M T Q S K F M S T S V G D R>	
GTC AGC GTC ACC TGC AAG GCC AGT CAG AAT GTG GGT ACT AAT GTA GCC TGG TAT	100
CAG TCG CAG TGG ACG TTC CGG TCA GTC TTA CAC CCA TGA TTA CAT CGG ACC ATA	
V S V T C K A S Q N V G T N V A W Y>	
CAA CAG AAA CCA GGA CAA TCT CCT AAA GCA CTG ATT TAC TCG GCA TCC TTC CTA	150
GTT GTC TTT GGT CCT GTR AGA GGA TTT CGT GAC TAA ATG AGC CGT AGG AAG GAT	
Q Q K P G Q S P K A L I Y S A S F L>	
TAT AGT GCA GTC CCT TAT CGC TTC ACA GGG AGT GGA TCT GGG ACA GAT TTC ACT	200
ATA TCA CCT CAG GGA ATA GCG AAG TGT CCG TCA CCT AGA CCC TGT CTA AAG TGA	
Y S G V P Y R F T G S G T D F T>	
110 120 130 140 150 160	210
TAT AGT GCA GTC CCT TAT CGC TTC ACA GGG AGT GGA TCT GGG ACA GAT TTC ACT	
ATA TCA CCT CAG GGA ATA GCG AAG TGT CCG TCA CCT AGA CCC TGT CTA AAG TGA	
Y S G V P Y R F T G S G T D F T>	
170 180 190 200 210	270
TAT AGT GCA GTC CCT TAT CGC TTC ACA GGG AGT GGA TCT GGG ACA GAT TTC ACT	
ATA TCA CCT CAG GGA ATA GCG AAG TGT CCG TCA CCT AGA CCC TGT CTA AAG TGA	
Y S G V P Y R F T G S G T D F T>	
220 230 240 250 260 310 320	270
CTC ACC ATC AGC ACT GTC CAG TCT GAA GAC TTG GCA GAG TAT TTC TGT CAG CAA	
GAG TGG TAG TCG TGA CAC GTC AGA CTT CTG AAC CGT CTC ATA AAG ACA GTC GTT	
L T I S T V Q S E D L A E Y F C Q Q>	
280 290 300	320
TAT AAC ATC TAT CCT CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA CGT	
ATA TTG TAG ATA GGA GAG TGC AAG CCA CGA CCC TGG TTC GAC CTC GAC TTT GCA	
Y N I Y P L T F G A G T K L E L K R>	

FIG.3 Murine V_H Sequence of hTNF40 (SEQ ID NO: 100)

FIG. 4
Comparisons of framework regions of light chain of
antibody hTNF40 and human group 1 consensus sequences

Hu group 1 consensus : DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 83)
hTNF40 : DIVMTQSOKEWMSSTSVGDRVSYTC (SEQ ID NO: 84)

Hu Group 1 consensus : WYQQKPGKAPKLLY (SEQ ID NO: 85)
hTNF40 : WYQQKPGOSPKALIY (SEQ ID NO: 86)

Hu Group 1 consensus : GVPSSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO: 87)
hTNF40 : GVPYRFTGSGSGTDFTLTISTYQSEDLAEYC (SEQ ID NO: 88)

Hu Group 1 consensus : FGQGTRKEIKR (SEQ ID NO: 89)
hTNF40 : FGAGTKLELKR (SEQ ID NO: 90)

FIG. 5

Comparisons of framework regions of heavy chain of antibody hTNF40 and human group 1 and group 3 consensus sequences

Hu Group 1 consensus : QVQLVQSGAEVKPGASVKVSCKASGYTFT (SEQ ID NO: 91)
hTNF40 : QIQLVQSGPELKKPGETVKISCKASGYVFT (SEQ ID NO: 92)

Hu Group 1 consensus : WVRQAPGQGLEWMG (SEQ ID NO: 93)
hTNF40 : WVKQAPGKAFKWMG (SEQ ID NO: 94)

Hu Group 1 consensus : RVTITRDTSTSTAYMELSSLRSEDTVYYCAR (SEQ ID NO: 95)
hTNF40 : RFAFSLETSASTAFLOINNLKNEDTATYFCAR (SEQ ID NO: 96)

Hu Group 1 consensus : WGQGTLVTVSS (SEQ ID NO: 97)
hTNF40 : WGQGTTLTVSS (SEQ ID NO: 98)

Hu Group 3 consensus : EVQLVESGGGLVQPGSLRLSCAASGFTFS (SEQ ID NO: 106)
hTNF40 : QIQLVQSGPELKKPGETVKISCKASGYVFT (SEQ ID NO: 92)

Hu Group 3 consensus : WVRQAPGKGLEWVS (SEQ ID NO: 107)
hTNF40 : WVKQAPGKAFKWMG (SEQ ID NO: 94)

Hu Group 3 consensus : RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR (SEQ ID NO: 108)
hTNF40 : RFAFSLETSASTAFLOINNLKNEDTATYFCAR (SEQ ID NO: 96)

Hu Group 3 consensus : WGQGTLVTVSS (SEQ ID NO: 109)
hTNF40 : WGQGTTLTVSS (SEQ ID NO: 98)

FIG. 6

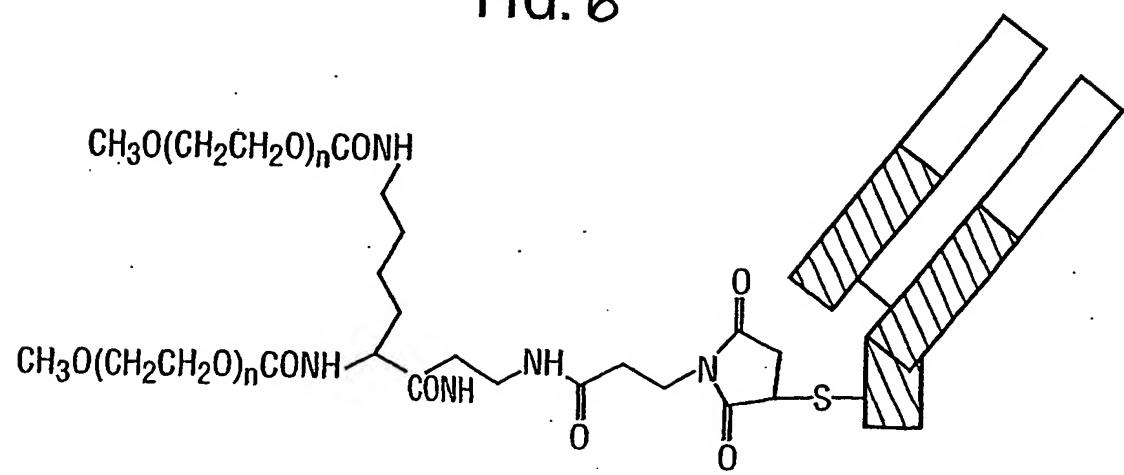


FIG. 7

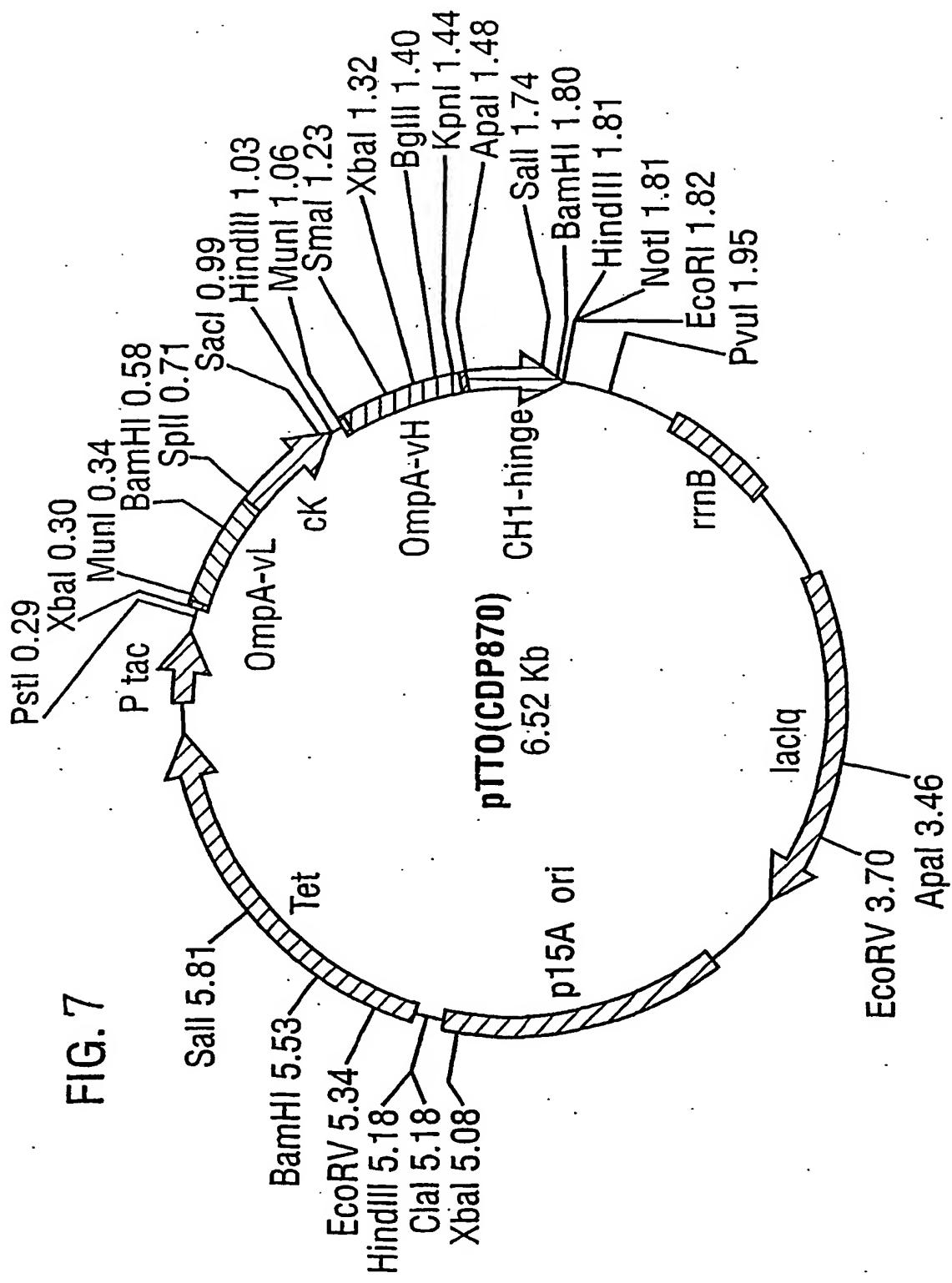
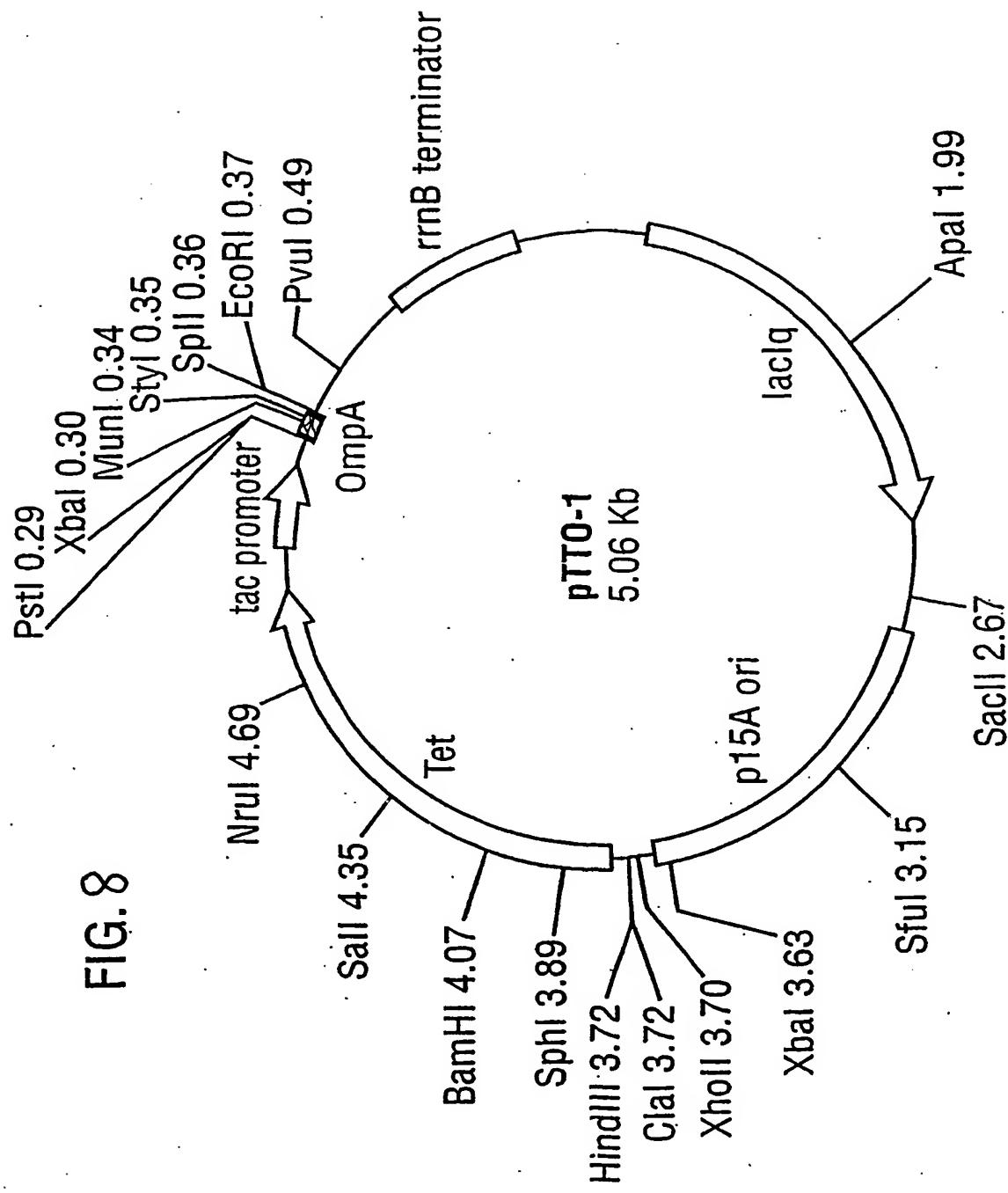


FIG. 8



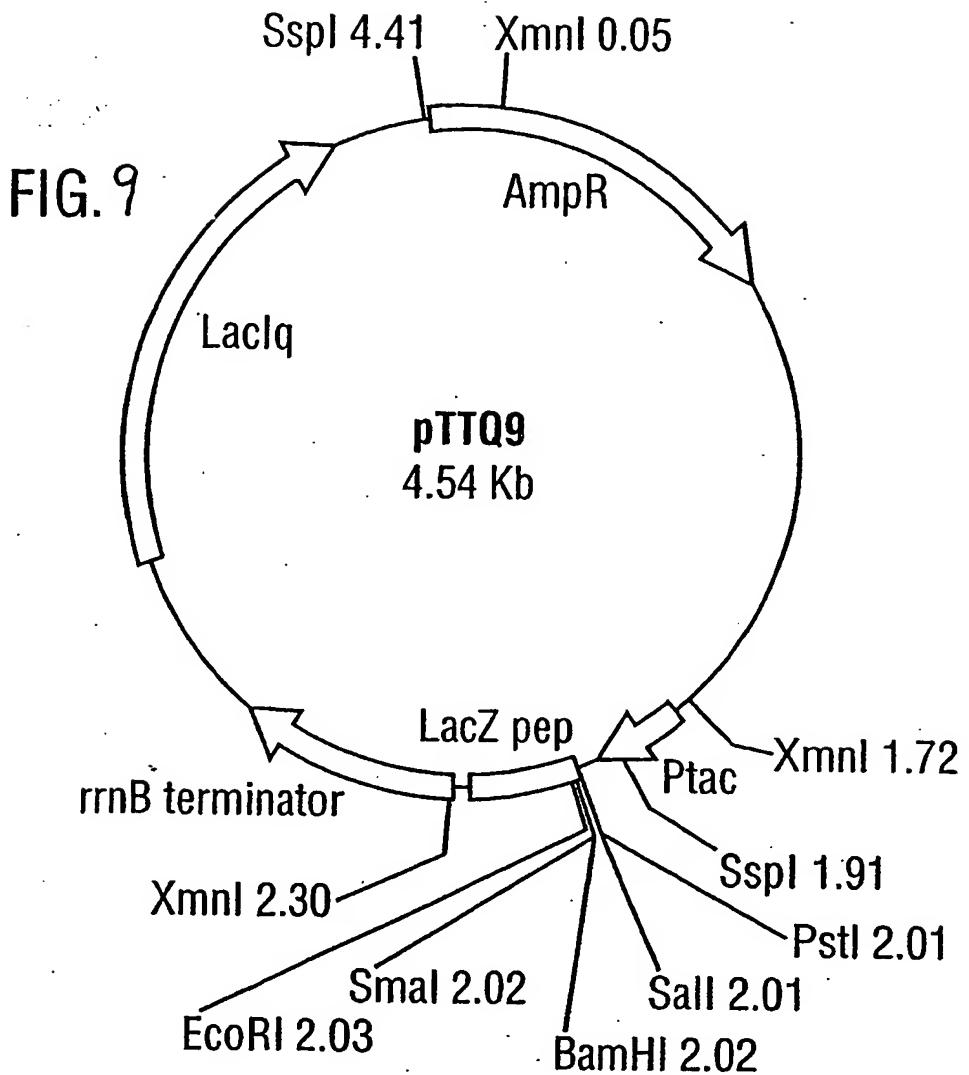


FIG. 10

Sequence of OmpA Oligonucleotide Adapter (SEQ ID NO: 101)

OmpA Leader

→

10	20	30	40
*	*	*	*
XhoI	XbaI	S.D.	

T CGA GTT CTA GAT AAC **GAG** GCG TAA AAA ATG AAA AAG ACA
CAA GAT CTA TTG CTC CGC ATT TTT TAC TTT TTC TGT
M K K T>

50	60	70	80
*	*	*	*
MunI	StyI	SplI	

GCT ATC GCA **ATT** GCA GTG GCC TTG GCT CTG ACG **TAC** GAG TCA
CGA TAG CGT **TAA** CGT CAC CGG AAC CGA GAC TGC **ATG** CTC AGT
A I A I A V A L A

90

*

EcoRI

GG

CCT TAA

- Internal restriction sites are shown in bold
- The 5' Xhol cohesive end ligates into the Vector Sall site, blocking it
- S.D. represents the OmpA Shine Dalgarno sequence

FIG. II

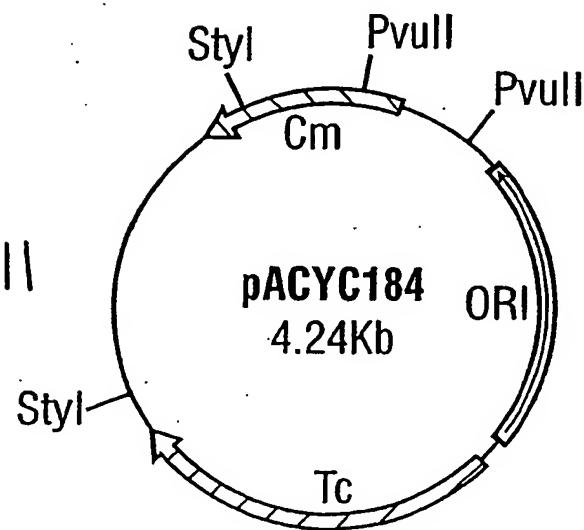


FIG. 12

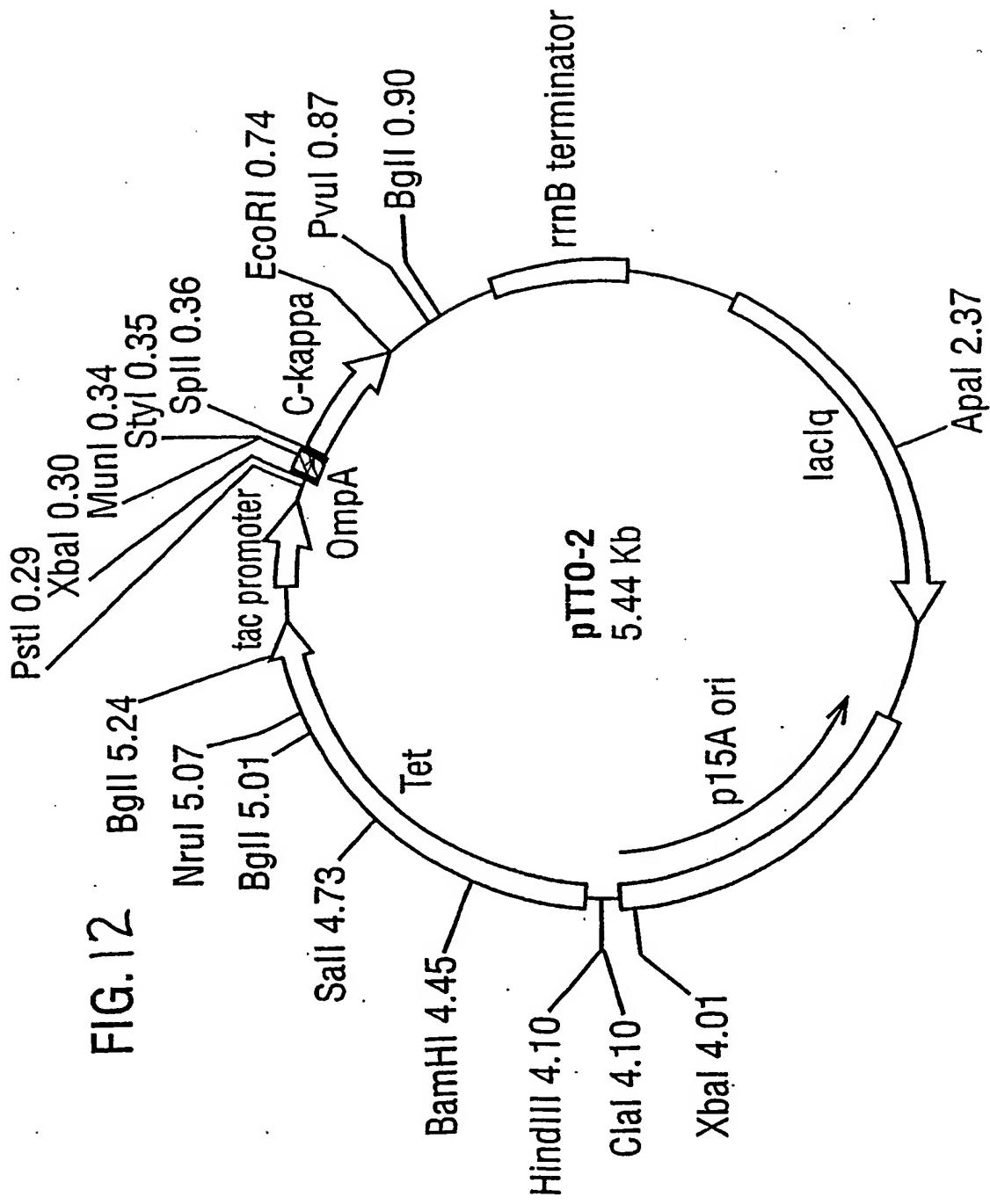


FIG. 13

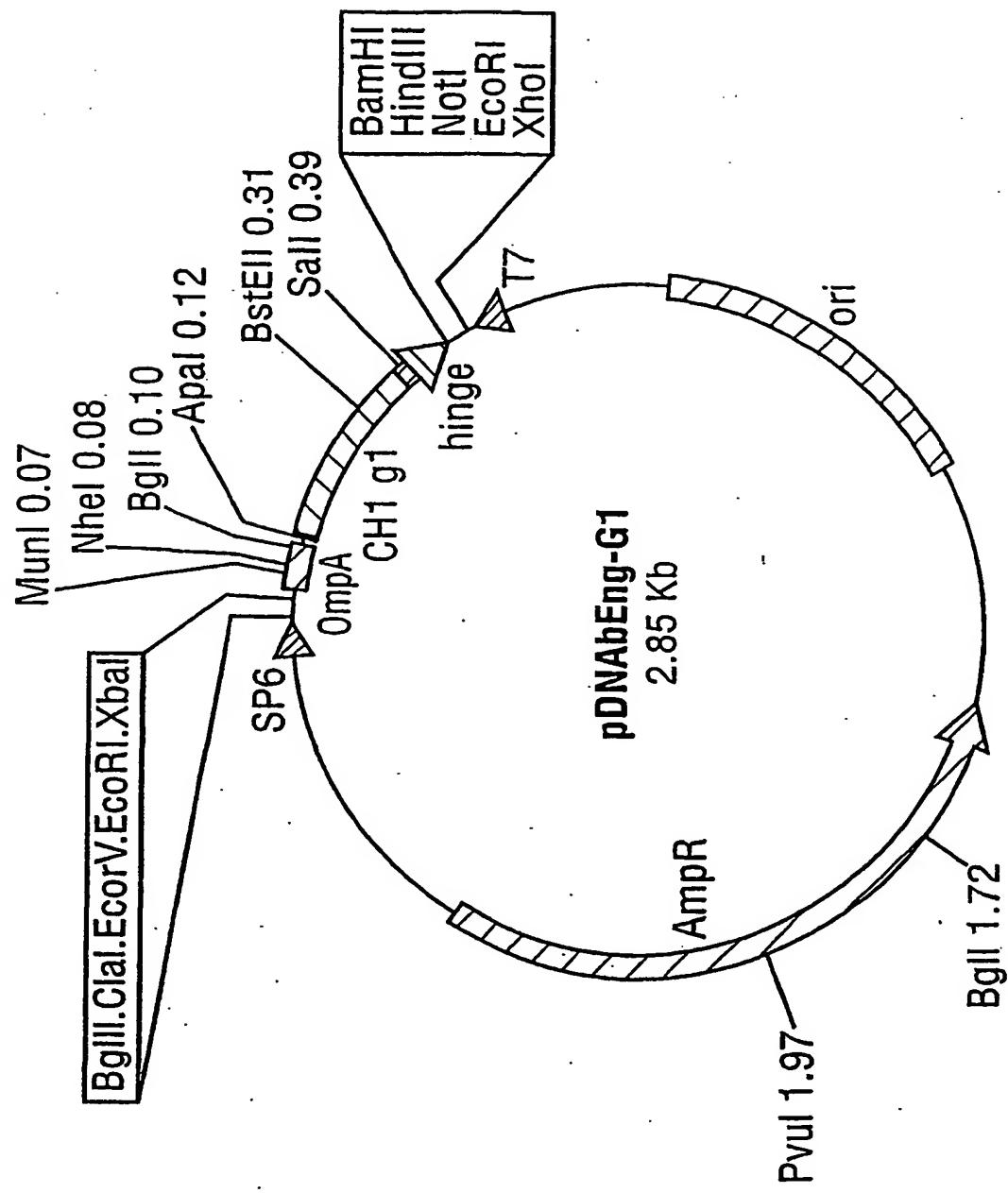


FIG. 14 OLIGONUCLEOTIDE CASSETTES ENCODING DIFFERENT INTERGENIC SEQUENCES FOR E. Coli Fab' EXPRESSION

IGS CASSETTE-1;

Intergenic space = -1

G, AGC, TCA, CCA, GCA, AAA, AGT, TTT, AAT, AGA, GGA, GAG, TGT, TATG, AAG, ACT, GCT, ATA, GCA, ATT, G (SEQ ID No: 102)

S S P V T K S F N R G E C * M K K T A I A I
End of c-Kappa sequence ->
Start of OmpA sequence ->

IGS CASSETTE-2;

Intergenic space = +1

G, AGC, TCA, CCA, GCA, AAA, AGT, TTT, AAT, AGA, GGA, GAG, TGT, TAA AATG, AAG, ACT, GCT, ATA, GCA, ATT, G (SEQ ID No: 103)

S S P V T K S F N R G E C * M K K T A I A I

IGS CASSETTE-3;

Intergenic space = +13

G, AGC, TCA, CCA, GCA, AAA, AGC, TTT, AAT, AGA, GGA, GAG, TGT, TGA GGGGAAMMMATG, AAG, AAA, ACT, GCT, ATA, GCA, ATT, G (SEQ ID No: 104)

S S P V T K S F N R G E C * M K K T A I A I

IGS CASSETTE-4;

Intergenic space = +13

G, AGC, TCA, CCA, GCA, ACA, AAA, AGT, TTT, AAT, AGA, GGA, GAG, TGT, TGA CGAGGATTATAATG, AAG, AAA, ACT, GCT, ATA, GCA, ATT, G (SEQ ID No: 105)

S S P V T K S F N R G E C * M K K T A I A I

FIG. 15 Grafted Vh Sequence of hTNF40.4 (SEQ ID NO: 11)

Figure 16

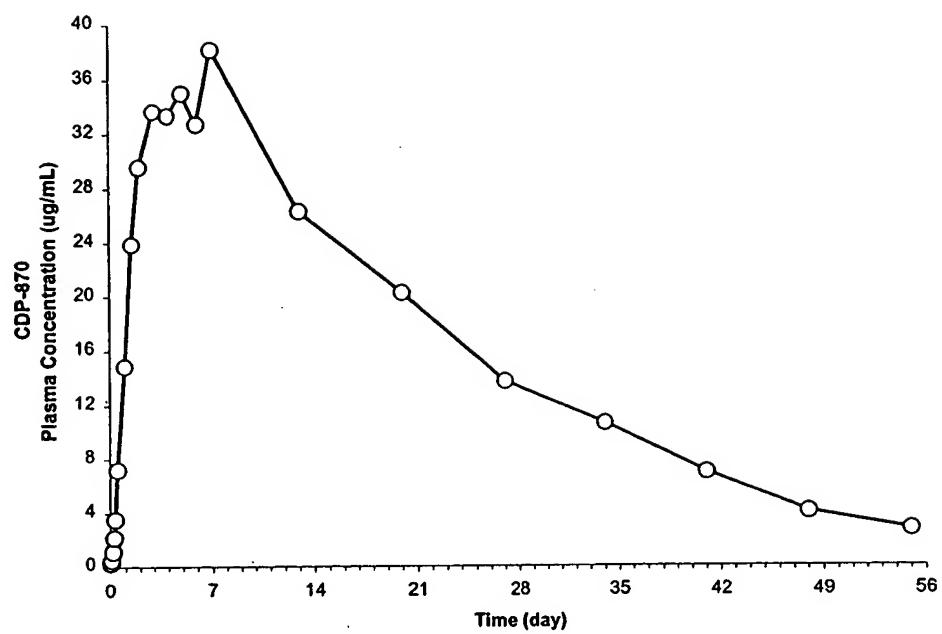


Figure 17

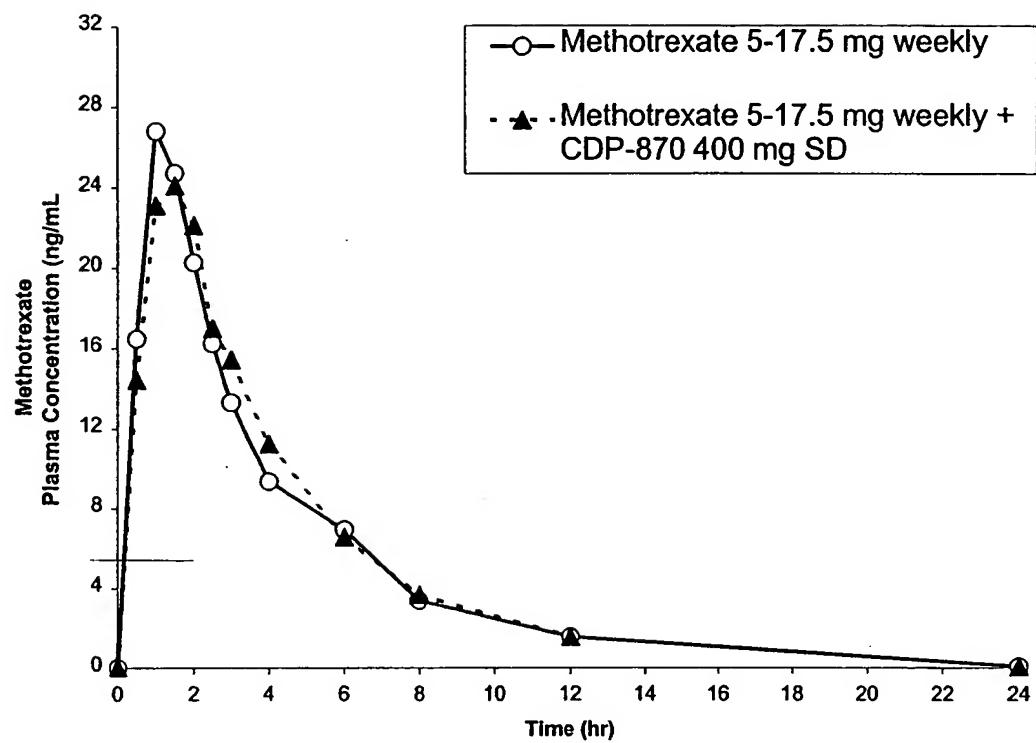


FIG. 18 CDP870 Heavy Chain mature protein sequence (SEQ ID No: 115)

EVQLVESGGGLVQPGGSLRLSCAASGYVFTDYGMNWVRQAPGKGLEWMGWINTYIGE
PIYADSVKGRFTFSLDTKSTAYLQMNSLRAEDTAVYYCARGYRSYAMDYWGQGTLVTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNSGALTSGVHTFPAVLQS SGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCAA*

FIG. 19 CDP870 Light Chain mature protein sequence (SEQ ID No: 113)

DIQMTQSPSSLSASVGDRVITCKASQNVGTNVAWYQQKPGKAPKALIYSASFYSGVP
YRFSGSGSGTDFLTISLQPEDFATYYCQQYNIYPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK
SGTASVVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC*

FIG. 20
(SEQ ID NOS: 116 – 117)

CDP870 nucleic acid sequences (shown as sense and anti-sense strands):

10	20	30	40	50
ATG AAA AAG ACA GCT ATC GCA ATT GCA GTG GCC TTG GCT GGT TTC GCT ACC ACC GTA				
TAC TTT TTC TGT CGA TAG CGT TAA CGT CAC CGG AAC CGA CCA AAG CGA TGG CAT				
60	70	80	90	100
GCG CAA GCT GAC ATT CAA ATG ACC CAG AGC CCA TCC AGC CTG AGC GCA TCT GTA				
CGC GTT CGA CTG TAA GTT TAC TGG GTC TCG GGT AGG TCG GAC TCG CGT AGA CAT				
110	120	130	140	150
GGA GAC CGG GTC ACC ATC ACT TGT AAA GCC AGT CAG AAC GTA GGT ACT AAC GTA				
CCT CTG GCC CAG TGG TAG TGA ACA TTT CGG TCA GTC TTG CAT CCA TGA TTG CAT				
170	180	190	200	210
GCC TGG TAT CAG CAA AAA CCA GGT AAA GCC CCA AAA GCC CTC ATC TAC AGT GCC				
CGG ACC ATA GTC GTT TTT GGT CCA TTT CGG GGT TTT CGG GAG TAG ATG TCA CGG				
220	230	240	250	260
TCT TTC CTC TAT AGT GGT GTA CCA TAC AGG TTC AGC GGA TCC GGT AGT GGT ACT				
AGA AAG GAG ATA TCA CCA CAT GGT ATG TCC AAG TCG CCT AGG CCA TCA CCA TGA				
280	290	300	310	320
GAT TTC ACC CTC ACG ATC AGT AGC CTC CAG CCA GAA GAT TTC GCC ACT TAT TAC				
CTA AAG TGG GAG TGC TAG TCA TCG GAG GTC GGT CTT CTA AAG CGG TGA ATA ATG				
330	340	350	360	370
TGT CAA CAG TAT AAC ATC TAC CCA CTC ACA TTC GGT CAG GGT ACT AAA GTA GAA				
ACA GTT GTC ATA TTG TAG ATG GGT GAG TGT AAG CCA GTC CCA TGA TTT CAT CTT				
380	390	400	410	420
ATC AAA CGT ACG GTA GCG GCC CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG				
TAG TTT GCA TGC CAT CGC CGG GGT AGA CAG AAG TAG AAG GGC GGT AGA CTA CTC				
440	450	460	470	480
CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CTC				
GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC ACG GAC GAC TTA TTG AAG ATA GGG				
490	500	510	520	530
AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC				
TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG GTT AGC CCA TTG AGG				
550	560	570	580	590
CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC				
GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG TCG TGG ATG TCG GAG TCG TCG				

FIG. 20 (cont'd)
 (SEQ ID NOS: 116 – 117)

CDP870 nucleic acid sequences (shown as sense and anti-sense strands):

600	610	620	630	640	
ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA TGG GAC TGC GAC TCG TTT CGT CTG ATG CTC TTT GTG TTT CAG ATG CGG ACG CTT					
650	660	670	680	690	700
GTC ACC CAT CAG GGC CTG AGC TCA CCA GTA ACA AAA AGC TTT AAT AGA GGA GAG CAG TGG GTA GTC CCG GAC TCG AGT GGT CAT TGT TTT TCG AAA TTA TCT CCT CTC					
710	720	730	740	750	
TGT TGA GG AGGAAAAAAA A ATG AAG AAA ACT GCT ATA GCA ATT GCA GTG GCG CTA ACA ACT CC TCCTTTTTT T TAC TTC TTT TGA CGA TAT CGT TAA CGT CAC CGC GAT					
760	770	780	790	800	810
GCT GGT TTC GCC ACC GTG GCG CAA GCT GAG GTT CAG CTG GTC GAG TCA GGA GGC CGA CCA AAG CGG TGG CAC CGC GTT CGA CTC CAA GTC GAC CAG CTC AGT CCT CCG					
820	830	840	850	860	
GGT CTC GTG CAG CCT GGC GGA TCA CTG AGA TTG TCC TGT GCT GCA TCT GGT TAC CCA GAG CAC GTC GGA CCG CCT AGT GAC TCT AAC AGG ACA CGA CGT AGA CCA ATG					
870	880	890	900	910	
GTC TTC ACA GAC TAT GGA ATG AAT TGG GTT AGA CAG GCC CCG GGA AAG GGC CTG CAG AAG TGT CTG ATA CCT TAC TTA ACC CAA TCT GTC CGG GGC CCT TTC CCG GAC					
920	930	940	950	960	970
GAA TGG ATG GGT TGG ATT AAT ACT TAC ATT GGA GAG CCT ATT TAT GCT GAC AGC CTT ACC TAC CCA ACC TAA TTA TGA ATG TAA CCT CTC GGA TAA ATA CGA CTG TCG					
980	990	1000	1010	1020	
GTC AAG GGC AGA TTC ACG TTC TCT CTA GAC ACA TCC AAG TCA ACA GCA TAC CTC CAG TTC CCG TCT AAG TGC AAG AGA GAT CTG TGT AGG TTC AGT TGT CGT ATG GAG					
1030	1040	1050	1060	1070	1080
CAA ATG AAT AGC CTG AGA GCA GAG GAC ACC GCA GTG TAC TAT TGT GCT AGA GGA GTT TAC TTA TCG GAC TCT CGT CTC CTG TGG CGT CAC ATG ATA ACA CGA TCT CCT					
1090	1100	1110	1120	1130	
TAC AGA TCT TAT GCC ATG GAC TAC TGG GGC CAG GGT ACC CTA GTC ACA GTC TCC ATG TCT AGA ATA CGG TAC CTG ATG ACC CCG GTC CCA TGG GAT CAG TGT CAG AGG					
1140	1150	1160	1170	1180	
TCA GCT TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC AGT CGA AGG TGG TTC CCG GGT AGC CAG AAG GGG GAC CGT GGG AGG AGG TTC TCG					

FIG. 20 (cont'd)
(SEQ ID NOS: 116 – 117)

CDP870 nucleic acid sequences (shown as sense and anti-sense strands):

1190	1200	1210	1220	1230	1240
ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA					
TGG AGA CCC CCG TGT CGC CGG GAC CCG ACG GAC CAG TTC CTG ATG AAG GGG CTT					
1250	1260	1270	1280	1290	
CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC					
GGC CAC TGC CAC AGC ACC TTG AGT CCG CGG GAC TGG TCG CCG CAC GTG TGG AAG					
1300	1310	1320	1330	1340	1350
CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG					
GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG CAC CAC TGG CAC					
1360	1370	1380	1390	1400	
CCC TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC					
GGG AGG TCG TCG AAC CCG TGG GTC TGG ATG TAG ACG TTG CAC TTA GTG TTC GGG					
1410	1420	1430	1440	1450	
AGC AAC ACC AAG GTC GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC					
TCG TTG TGG TTC CAG CTG TTC TTT CAA CTC GGG TTT AGA ACA CTG TTT TGA GTG					
1460	1470				
ACA TGC GCC GCG TGA TGA					
TGT ACG CGG CGC ACT ACT					